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IS 6387 (1987): Specification for vegetable Protein Infant Food With Milk [FAD 8: Food Additives]

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AMENDMENT NO. 1 JUNE 1996
TO
IS 6387 : 1987 SPECIFICATION FOR VEGETABLE PROTEIN INFANT FOOD WITH MILK

(First Revision)

(*Page* 1, *clause* **0.3**) — Insert the following new clause after **0.3**:

'**0.4** A scheme for labelling environment friendly products known as ECO-Mark has been introduced at the instance of the Ministry of Environment and Forests (MEF), Government of India. The ECO-Mark shall be administered by the Bureau of Indian Standards (BIS) under the *BIS Act*, 1986 as per the Resolution No. 71 dated 20 February 1991 and Resolution No. 425 dated 28 October 1992 published in the Gazette of the Government of India. For a product to be eligible for marking with the ECO-Mark it shall also carry the Standard Mark of BIS for quality besides meeting additional environment friendly (EF) requirements. The environment friendly requirements for vegetable protein infant food with milk are, therefore, included through Amendment No. 1 to this standard.

This amendment is based on the Gazette Notification No. 624 (E) dated 6 September 1995 for Labelling Beverages, Infant Foods, Processed Fruits and Vegetable Products as environment friendly, published in the Gazette of the Government of India.'

(*Page* 4, *sub-clause* 3.3.7) — Insert the following new clauses after **3.3.7**:

''**3.4 Additional Requirements for ECO-Mark**

3.4.1 *General Requirements*

3.4.1.1 The product shall conform to the requirements prescribed under **3.1** to **3.3.7**.

3.4.1.2 The manufacturer shall produce the consent clearance as per the provisions of *Water (PCP) Act*, 1974, *Water (PCP) Cess Act*, 1977 and *Air (PCP) Act*, 1981 along with the authorization if required under *Environment (Protection) Act*, 1986 and the Rules made thereunder to the Bureau of Indian Standards while applying for the ECO-Mark and the product shall also be in accordance with the *Prevention of Food Adulteration Act*, 1954 and the Rules made thereunder. Additionally, FPO 1955 (Fruit Product Order) framed under *Essential Commodities Act*, 1955, *Standards of Weights and Measures Act*, 1977 requirements wherever applicable has to be complied with.

3.4.1.3 The product/packaging may also display in brief the criteria based on which the product has been labelled environment friendly.

3.4.1.4 The material used for product/packing shall be recyclable or biodegradable.

3.4.1.5 The date of manufacture and date of expiry shall be declared on the product/package by the manufacturer.

3.4.1.6 The product shall be microbiologically safe when tested as per IS 5403 : 1969 'Method for yeast and mould count of foodstuffs ' and IS 5887 (Part 5) : 1976 'Methods for detection of bacteria responsible for food poisoning : Part 5 Isolation, identification and enumeration of *Vibrio Cholerae* and *Vibrio Parahaemolyticus* (*first revision*)' and shall be free from bacterial and fungal toxins.

3.4.1.7 The pesticide residues, if any in the product shall not exceed the limit as prescribed in *PFA Act*, 1954 and the Rules made thereunder.

3.4.1.8 The product/package or leaflet accompanying it may display instructions of proper use, storage and transport (including refrigeration temperature compliance) so as to maximize the product performance, safety and minimize wastage.

3.4.2 *Specific Requirements*

3.4.2.1 Arsenic content of the product shall not exceed 0.1 mg/kg when tested in accordance with the method given in IS 11124 : 1984 'Method for atomic absorption spectrophotometric determination of arsenic'.

3.4.2.2 The material used inside the metal cap of the product shall conform to the relevant Indian Standards of food grade plastics as permitted under the *Prevention of Food Adulteration Act*, 1954 and the Rules made thereunder. Caps and closures shall not be treated as labels.

3.4.2.3 The percentage of fruit juice/pulp, if any added shall be mentioned on the product/package.

3.4.2.4 No synthetic food colour and artificial sweetener shall be added or used in the product.

3.4.2.5 Product shall be free from aflatoxins when tested in accordance with the method prescribed in Appendix J of IS 4684 : 1975 'Specification for edible groundnut flour (expeller pressed) (*first revision*)'."

(*Page* 5, *clause* **4.2**) — Insert the following new clause after **4.2**:

'4.3 ECO-Mark

The product may also be marked with the ECO-Mark, the details of which may be obtained from the Bureau of Indian Standards.'

(FAD 57)

UDC 637·144 : 664·38

(Reaffirmed 2003)

Indian Standard

SPECIFICATION FOR
VEGETABLE PROTEIN INFANT FOOD WITH MILK

(First Revision)

0. Foreword

0.1 Human milk ideally fulfils the entire requirement for growth and additionally provides unique bio-immune factors for protecting the health of infants. Breast feeding is, therefore, universally regarded as the most appropriate form of milk nourishing the infant. However, when breast feeding is not possible, reliance has to be placed upon the alternate sources of nutrients for infant feeding. It is imperative that infant formula should be properly formulated so that nutritional requirements for optimal growth are met adequately, and that there is minimum of physiological stress on the developing organ/enzymic system of the infant. It is equally important to promote correct feeding practices so that appropriate use of the infant food could be made for protecting the health of the infant.

0.2 Infant foods at present marketed in our country could be divided into five main categories: (a) infant milk foods, (b) infant formulae, (c) vegetable protein infant foods with milk, (d) milk-cereal based weaning foods, and (e) processed cereal-based complementary foods. This standard covers only vegetable protein infant foods with milk which are intended for the partial or total replacement of breast milk.

0.3 While formulating this standard, due consideration has been given to the relevant rules prescribed by the Government of India under the Prevention of Food Adulteration Act, 1954 and the Standard of Weights and Measures (Packaged Commodities) Rules, 1977. This standard is, however, subject to the restrictions imposed under these, wherever applicable.

1. Scope — This standard prescribes the requirements and the methods of sampling and test for vegetable protein infant food with milk (hereafter referred to as infant food).

2. Terminology — For the purpose of this standard, the following definitions shall apply.

2.1 *Vegetable Protein Infant Food with Milk* — The material prepared by drying using a suitable method, such as spray or roller drying an emulsion of vegetable proteins, vegetable oils rich in polyunsaturated fatty acids and milk with added carbohydrates or by blending the components. The vegetable proteins may be derived from cereals, pulses or legumes, singly or in a combination thereof. The infant food contains a minimum of 25 percent non-fat milk solids. The carbohydrates used may be sucrose, dextrose and dextrins, maltose and lactose. Salts like phosphates and citrates, vitamin A and all other essential vitamins, and minerals like calcium and iron shall also be added. Other optional minerals and vitamins may also be added.

2.2 *Infant* — A child not more than 12 months of age.

2.3 *Routine Tests* — Tests (*see* **6**) carried out on each lot to check the essential requirements that are likely to vary during production.

2.4 *Type Tests* — All the tests (*see* **3.3**) to approve the design and quality of the product at least in the beginning of marketing or certification or both. These tests may also be conducted periodically to supplement the routine tests or whenever the basic formula is changed.

3. Requirements

3.1 *General Requirements*

3.1.1 *Quality of ingredients* — All ingredients including the optional ones shall be clean, of good quality, safe and suitable for ingestion by infants. They shall conform to their normal quality requirements, such as colour, flavour and odour.

Dairy Products Sectional Committee, AFDC 34 [Ref : Doc : AFDC 34 (2860)]

Adopted 31 December 1987

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Gr 4

BUREAU OF INDIAN STANDARDS
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NEW DELHI 110002

3.1.1.1 The vitamins and minerals shall be of food grade. Iron or iron salts should be such as to ensure high bio-availability of iron. The source of iron may be selected from ferrous citrate, ferrous fumarate, ferrous succinate, ferrous sulphate, ferric ammonium citrate and ferric pyrophosphate.

3.1.1.2 The vegetable oils and protein rich flours used shall conform to the relevant Indian Standards, wherever available.

3.1.1.3 All processing and drying should be carried out in a manner that minimizes the loss of nutritive value, particularly protein quality.

3.1.1.4 The moisture content of the product shall be governed by good manufacturing practices and shall be at such a level that there is minimum loss of nutrition value and at which micro-organisms cannot multiply.

3.1.2 *Optional ingredients* — In addition to the vitamins and minerals given in Table 1, other nutrients given in Table 2 may be added in order to provide nutrients ordinarily found in human milk and to ensure that the infant formula is suitable as the sole source of nutrients of the infants. When any of these nutrients are added, the infant formula shall contain significant amount of these nutrients, based on their levels in human milk. Ascorbyl palmitate up to a maximum of 1 mg/100 ml of the reconstituted (ready-to-drink) feed may also be added.

TABLE 1 COMPOSITIONAL SPECIFICATION FOR VEGETABLE PROTEIN INFANT FOOD WITH MILK

Sl No.	Characteristic	Requirement per		Method of Test, Ref to	
		100 g (percent by mass)	100 available kilojoules (kJ) [100 available kilocalories (kcal)]	Appendix of IS : 1547-1985*	Other Indian Standards
(1)	(2)	(3)	(4)	(5)	(6)
i)	Moisture, g, *Max*	4·5	—	A	—
ii)	Total protein, g, *Min*	14·0	0·75(3·16)	—	IS : 7219-1973†
iii)	Total fat, g, *Min* (*see* **3.2.1**)	18·0	0·99(4·14)	B	—
iv)	Total ash, g, *Max*	6·0	0·33(1·38)	C	—
v)	Acid insoluble ash, g, *Max*	0·10	0·005(0·02)	D	—
vi)	Crude fibre (on dry basis), g, *Max*	1·0	—	—	IS : 10226 (Part 1)-1982‡
vii)	Solubility:				
	a) Solubility index, ml, *Max* or	15·0 (if roller dried) 2·0 (if spray dried)	—	E	—
	b) Solubility, g, *Min*	85·0 (if roller dried) 98·5 (if spray dried)	—	F	—
viii)	Vitamin A (as retinol), µg, *Min*	350	17·74(75)	—	IS : 5886-1970§
ix)	Added vitamin D (expressed as cholescalciferol) IU, *Min*	180	9·55(40)	—	IS : 5835-1970‖ (*see* Note 3)
x)	Iron, mg, *Min*	5·0	0·275(1·15)	G	—

Note 1 — 1 kJ = 0·238 846 kcal or 1 kcal = 4·186 8 kJ.

Note 2 — For conversion of the values of requirements of the characteristics from percent by mass (per 100 g) to the value per 100 kcal, the total kcal content of the food shall be based upon the values of 4 kcal per g of carbohydrates, 4 kcal per g of proteins and 9 kcal per g of fat. The total carbohydrates are calculated by the difference as given in Appendix H of IS : 1547-1985*.

Note 3 — As there is no suitable and easily workable method for determining vitamin D content of a product like the infant formula, the manufacturers shall be required to maintain a record showing the quantity of vitamin D added to each batch. For this purpose, the vitamin D content of the infant milk food may be calculated on the basis of the vitamin D content as declared by the manufacturer of the vitamin D preparation used for vitaminization.

Note 4 — Requirements are on 'as is' basis.

*Specification for infant milk foods (*second revision*).

†Method for determination of protein in foods and feeds.

‡Method for determination of crude fibre content in food products: Part 1 General method.

§Methods for estimation of carotenes and vitamin A (retinol) in foodstuffs.

‖Method for estimation of vitamin D in foodstuffs.

TABLE 2 OPTIONAL NUTRIENTS

(*Clause* 3.1.2)

Sl No.	Characteristic	Recommended Level per		Method of Test, Ref to
		100 g (percent by mass)	100 available kilojoules (kJ) [100 available kilocalories (kcal)]	
(1)	(2)	(3)	(4)	(5)
i)	Thiamine, µg, *Min*	185	10·15(42·55)	IS : 5398-1969[1]
ii)	*Nicotinamide, µg, *Min*	1 160	63·70(266·80)	IS : 5400-1969[2]
iii)	Riboflavin, µg, *Min*	275	15·10(63·25)	IS : 5399-1969[3]
iv)	Vitamin B_6, µg, *Min*	160	8·80(36·80)	IS : 7530-1975[4]
v)	Vitamin B_{12}, µg, *Min*	0·7	0·04(0·16)	IS : 7529-1975[5]
vi)	Folic acid, µg, *Min*	20	1·10(4·60)	IS : 7234-1974[6]
vii)	Pantothenic acid, mg, *Min*	1·4	0·08(0·32)	IS : 9840-1981[7]
viii)	Biotin, µg, *Min*	7·0	0·38(1·61)	IS : 9820-1971[8]
ix)	Vitamin C, mg, *Min*	35	1·90(8·05)	IS : 5838-1970[9]
x)	Vitamin K, µg, *Min*	18	1·0(4·10)	—
xi)	Copper, µg, *Min*	280	15·38(64·40)	9 of IS : 7874 (Part 2)-1975[10]
xii)	Iodine, µg, *Min*	20	1·20(5·00)	8
xiii)	Manganese, µg, *Min*	20	1·20(5·00)	10
xiv)	Zinc, mg, *Min*	2·5	0·14(0·58)	13

[1] Methods for estimation of thiamine (vitamin B_1) in foodstuffs.
[2] Methods of estimation of nicotinic acid (niacin) in foodstuffs.
[3] Methods for estimation of riboflavin (vitamin B_2) in foodstuffs.
[4] Methods for estimation of pyridoxine (vitamin B_6) in foodstuffs.
[5] Method for estimation of vitamin B_{12} in foodstuffs.
[6] Method for estimation of folic acid in foodstuffs.
[7] Method for estimation of pantothenic acid in foodstuffs.
[8] Method for estimation of biotin in foodstuffs.
[9] Methods for estimation of vitamin C in foodstuffs.
[10] Methods of tests for animal feeds and feeding stuffs: Part 2 Minerals and trace elements.

*The nicotinamide per 100 g may be calculaled by multiplying the nicotinic acid (niacin) content per 100 g by 0·992.

3.2 *Routine Tests*

3.2.1 *Description* — The infant formula shall be white or white with a greenish tinge to light cream in colour, reasonably free from lumps, brown or scorched particles and shall be uniform in appearance. It shall be free from starch and added antioxidants [except ascorbyl palmitate, if added (*see* **3.1.2**)]. Vegetable oils rich in polyunsaturated fatty acids shall be added to partially substitute milk fat to an extent that the product shall contain a minimum of 12 percent by mass of milk fat and a minimum linoleate (linoleic acid in the form of glyceride) content of 1 398 mg/100 g [76·43/100 available kilojoules (kJ) or 320 mg/100 available kilocalories (kcal)] of the product when determined according to the method prescribed in Appendix A. The product shall also contain a minimum of 0·70 IU of vitamin E *dl*-α-tocopheryl acetate or 0·469 8 mg *d*-α-tocopherol per gram of linoleates when determined according to the method prescribed in IS : 7235-1974 'Method for estimation of tocopherols (vitamin E) in foodstuffs'. It shall also be free from dirt and extraneous matter, preservatives, added colour and flavour, and from any material and bacteria which are harmful to human health.

3.2.2 *Flavour* — The flavour of the infant formula in dry condition or of the reconstituted food shall be fresh and sweet. The infant formula shall not have rancid taste or musty odour.

3.2.3 *Hygienic conditions* — The material should be, as far as possible, manufactured and packed under hygienic conditions in licensed premises [*see* IS : 2491-1972 'Code for hygienic conditions for food processing units *(first revision)*'].

3.2.4 *Bacteriological specifications*

3.2.4.1 *Bacterial count* — The colony count per g of the product shall not be more than 40 000 when determined according to the method prescribed in IS : 5402-1969 'Method for plate count of bacteria in foodstuffs'.

3.2.4.2 *Coliform count* — The coliform bacteria shall be absent in 0·1 g of the product when determined according to the method prescribed in IS : 5401-1969 'Method for detection and estimation of coliform bacteria in foodstuffs'.

3.2.5 The infant food shall also comply with the specifications given in Table 1.

3.3 *Type Tests*

3.3.1 *Aflatoxin content* — When cottonseed or soya flours have been used in making the infant food, aflatoxin content shall be not more than 30 μg/kg of food, when tested according to the method prescribed in Appendix J of IS : 4684-1975 'Specification for edible groundnut flour (expeller pressed)'.

3.3.2 *Gossypol content* — When cottonseed has been used in making the infant food, the free gossypol content shall be not more than 0·065 percent by mass and total gossypol content not more than 1·10 percent by mass, when tested according to the method prescribed in Appendices B and C of IS : 4876-1986 'Specification for edible cottonseed flour (solvent extracted) (*first revision*)'.

3.3.3 *Urease activity* — When soya flour has been used, infant food shall not show a change in *p*H by more than 1·0 when determined by the method given in Appendix D of IS : 7837-1975 'Specification for edible full-fat soya flour.

3.3.4 *Protein efficiency ratio* — The infant food shall have minimum protein efficiency ratio (PER) of 2·4 with reference to protein efficiency ratio (PER) of casein as 2·5 and skimmed milk powder 3·0, when tested according to the method prescribed in IS : 7481-1974 'Method for determination of protein efficiency ratio (PER)'.

3.3.5 *Linoleic acid* — The infant food shall have a minimum linoleate (linoleic acid in the form of glyceride) content of 1 398 mg/100 g [76·43/100 available kilojoules (kJ) or 320 mg/100 available kilocalories (kcal)] when determined according to the method prescribed in Appendix A.

3.3.6 *Vitamin E* — The infant food shall contain a minimum of 0·70 IU of vitamin E *dl*-α-tocopheryl acetate or 0·469 8 mg *d*-α-tocopherol per gram of linoleates when determined according to the method prescribed in IS : 7235-1974 'Method for estimation of tocopherols (vitamin E) in foodstuffs'.

3.3.7 *Antitryptic activity* — When soya bean is the source of protein, there shall be no antitryptic activity in the product when determined according to the method prescribed in Appendix C of IS : 7837-1975.

4. Packing and Marking

4.1 The infant food shall be packed in hermetically sealed, clean and sound (*see* IS : 11078-1984 'Specification for round metal cans for milk powder) containers in quantities of 0·2, 0·4, 0·5 and 1 kg in such a way as to protect it from deterioration. It shall be packed in nitrogen or a mixture of nitrogen and carbon dioxide.

4.2 The containers shall bear legibly and indelibly the following in addition to the information necessary under the Indian National Code for Protection and Promotion of Breast-Feeding (Ministry of Social Welfare, Government of India):

a) Name of the material, that is, vegetable protein infant food with milk and the trade-name or brand name, if any;

b) Name and address of the manufacturer;

c) Batch or code number;

d) Net mass;

e) Process of manufacture (spray dried or roller dried or other);

f) Composition with special reference to any particular additions;

g) Directions for use;

h) Precautions against improper use (*see* **4.2.1**);

j) The date before which the contents should be used by marking the words 'To be used before.........';

k) Important Notice — Mother's milk is best for your baby; and

m) Any other requirements under the *Standards of Weights and Measures (Packaged Commodities) Rules, 1977.*

Note 1 — It is optional for the manufacturer to give such directions with reference to the age of the infant depending upon the protein level of the food.

Note 2 — The word 'pure' shall not be used in markings.

4.2.1 The following special precautions shall be suitably included either on the label or in accompanying leaflets:

a) The bottles and tests should be cleaned and sterilized by keeping in boiling water for a minimum of 5 minutes before using them for preparation of feed;

b) Exact quantities recommended for infant food and water shall be used for preparation of the feed. Over dilution/under dilution is harmful for infant health;

c) Once the feed is prepared, use it within half-an-hour otherwise microbial deterioration of the product may set in;

d) Discard unused feed;

e) Instructions for use of measuring scoop should be explicit (whether it should be level or heaped); and

f) The amounts of vitamins provided by the oral drops should take into account the amount of those provided through infant formulae so as to avoid overdosage.

4.2.2 *Standard marking* — Details available with the Bureau of Indian Standards.

5. Sampling — Representative samples of the material shall be drawn and tested for conformity to this standard as prescribed in Appendix J of IS : 1547-1985.

6. Tests

6.1 Tests shall be carried out as prescribed in the appropriate Appendices and Indian Standards given in col 5 and 6 of Table 1.

6.2 *Quality of Reagents* — Unless specified otherwise, pure chemicals and distilled water [*see* IS : 1070-1977 'Specification for water for general laboratory use (*second revision*)'] shall be employed in tests.

Note — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the results of analysis.

APPENDIX A

(*Clauses* 3.2.1 *and* 3.3.5)

METHOD OF DETERMINATION OF LINOLEIC ACID

A-0. General

A-0.1 The method is of special application to determination of linoleic and linolenic acids in products containing little or no other polyunsaturated acids.

A-1. Definition

A-1.1 Polyunsaturated fatty acids in which the double bonds are not conjugated are spectroscopically transparent in the ultra-violet region of the spectrum, that is, they show no absorption bonds. On conjugation, linoleic acid, linolenic acid and any higher unsaturated constituents show ultra-violet absorption bands at specific wavelengths. Provided the behaviour of pure samples of these acids under the same standardized conditions has been determined, the amount of each acid present can be calculated. The calculation takes into account the fact that in a mixture of linoleic and linolenic acids (or as esters and soaps) all the triple conjugation shall arise from the linolenic acid whereas the double conjugation arises from both linoleic and linolenic acids.

A-2. Principle

A-2.1 The fatty acid mixture under test is heated with a reagent prepared from glycerol and caustic potash under standard conditions, and the reacted mixture is diluted with alcohol to a fixed volume. The ultra-violet absorption of the solution is then measured in a spectrophotometer at 234 and 268 mμ and the linoleic and linolenic acids calculated.

A-3. Reagents

A-3.1 *Glycerol/Potassium Hydroxide Reagent* — Add 17·5 to 17·7 g potassium hydroxide to 100 ml (126 g) of glycerine and heat the mixture in an open stainless steel beaker to 200°C stirring with a thermometer to drive off water. Small pellet of potassium hydroxide is preferable and should be taken from a recently opened or a previously used but well-sealed bottle. On reaching 200°C, remove the source of heat and allow to cool. The strength should be 10·9 to 11·0 percent (*m/m*) potassium hydroxide. The reagent should always be freshly prepared before use.

A-3.2 *Ethyl Alcohol* — Obtained from industrial methylated spirit by fractional distillation over potassium hydroxide in a suitable fractionating column. Collect the distillate in three main fractions and reserve the middle fraction for use in the test, after having checked the transmittance at 234 to 268 mμ. Between 60 to 70 percent transmittance at these wavelengths is considered satisfactory.

A-4. Apparatus

A-4.1 *Suitable Spectrophotometer*

A-4.2 *Heat-Resistant Glass Boiling Tubes* — 15 × 2·5 cm.

A-4.3 *Oil-Bath* — capable of being maintained at 180 ± 0·5°C.

A-4.4 *Small Tubes* — about 20 × 10 mm, closed at one end (iodine value tubes).

A-4.5 *Solid Stem Thermometer* — reading up to 200°C.

A-4.6 *Graduated Flasks* — 50 ml.

A-5. Procedure

Sl No.	*Step*	*Precaution*	*Explanatory Note*
1)	Weigh 11 g glycerol/potassium hydroxide reagent into each of three 15 × 2·5 cm glass boiling tubes.	±0·01 g	Two of these are for duplicate determinations and the third a control.
2)	Immerse these in an oil-bath heated to 180 ± 0·5°C.	See that the liquid in the tubes is entirely covered.	—
3)	Take the temperature by means of a thermometer arranged so that the bulb lies centrally in the liquid in control tube.	The bath shall be steady at 180°C at the time of inserting the tubes.	Undue heating of the reagent promotes attack on the glass tubes, causing turbidity, and also affecting the precision of the test.
4)	Weigh accurately into each of two iodine value tubes about 0·1 g of fatty material.	—	—
5)	Place one of these each into two of the boiling tubes containing the reagent. Place an empty tube into the control.	—	—
6)	As the addition is made to an individual tube, remove it from the batch and swirl vigorously for 30 seconds.	—	With fatty acids, the saponification proceeds easily but if neutral glyceride oils are being tested, saponification does not take place readily, and low results may be obtained, caused by oxidation and polymerization at 180°C.

Sl No,	*Step*	*Precaution*	*Explanatory Note*
		—	If even a small amount of free fatty acid is present, the saponification proceeds satisfactorily, presumably owing to the immediate formation of a little soap which promotes emulsification of the phases.
		In case of difficulty experienced from this cause, add 0·1 g of pure stearic acid (± 0·05 g) in both the test control tubes.	Isomerization will then proceed smoothly.
7)	Return each tube to the bath and maintain it at 180°C for exactly 30 minutes.	—	—
8)	Remove each tube precisely at the end of this period, cool a little and add 10 to 20 ml alcohol.	—	—
9)	Swirl vigorously, while maintaining it in a warm condition.	—	—
10)	Transfer each solution to a 50-ml graduated flask, washing it with about 20 ml alcohol.	—	—
11)	Cool each flask to room temperature and make up to volume with alcohol.	—	—
12)	Dilute each suitably (*see* Table 3) with alcohol and with the control dilution in the reference cell. Determine the ultra-violet absorption over the range of 225-300 mμ.	Silica absorption cells shall be used. The whole curve is not essential but it is advisable to see that there are no abnormalities present if a material of unknown origin is being examined.	For samples containing large proportions of linoleic and/or linolenic acids, the diene peak maximum may be found to shift from 234 mμ to 232 or 231 mμ. Accordingly, peak maximum wavelength should be determined for such samples. Samples of marine origin will show peaks at 301, 315, 345 mμ and even longer wavelengths. The presence of acids causing these peaks renders the formulae given below invalid. The diene wavelength shift is very likely caused by formation of different mixtures of conjugated geometric isomers.
13)	Calculate percent linoleic and linolenic acids as indicated below.	—	—

Note — Natural fats (other than a few exception, such as, tung oil) contain no conjugated acids. The ultra-violet absorption of fatty acids derived from natural fats before isomerization is usually negligible and ultra-violet measurement need only be carried out after isomerization. In the case of tung oil fatty acids and certain processed oil fatty acids, for example, acids from partially hydrogenated oils where a measurable amount of conjugation is present, examination shall also be carried out before isomerization.

A-6. Calculation

A-6.1 Calculate the *E* (1 percent, 1 cm) values at 234 mμ (or the relevant diene peak maximum wavelength) and at 268 mμ as follows:

$$E\text{ (1 percent, 1 cm)} = \frac{\text{Observed optical density}}{C \times L}$$

where

C = concentration of sample solution in gram per 100 ml, and

L = light path length of absorption cell in centimetres.

A-6.1.1 Calculate the percent linoleic and linolenic acids according to the following:

Linoleic acid, percent = 0·113 *E* (1 percent, 1 cm) 234 mμ − 0·127 *E* (1 percent, 1 cm) 268 mμ

Linolenic acid, percent = 0·187 *E* (1 percent, 1 cm) 268 mμ

If results are required to be calculated to a glyceride basis, multiply by 1·05 in each case.

TABLE 3 DILUTIONS REQUIRED FOR ORIGINAL SAMPLE SOLUTION

(*Clause* A-5, *Sl No.* 12)

Sl No.	Material	For Reading at 234 mμ			For Reading at 268 mμ		
		Volume of Original Solution to be Taken	To be Diluted to	Percent Sample in Solution	Volume of Original Solution to be Taken	To be Diluted to	Percent Sample in Solution
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
i)	Bonegreases, tallows and derived fatty acids, oleines of iodine value less than 90	2 ml	25 ml	0·016	2 ml	25 ml	0·016
ii)	Groundnut oil and derived fatty acids, oleines of iodine value greater than 90	1 ml	25 ml	0·008	1 ml	25 ml	0·008
iii)	Linoleic acid and its esters, sunflowerseed, safflowerseed, tobacco seed, nigerseed oils, etc, and derived fatty acids	1 ml then 1 ml(A)	25 ml(A) 25 ml	0·000 64	2 ml	25 ml	0·016
iv)	Soyabean oil and derived fatty acids	2 ml then 1 ml(A)	25 ml(A) 25 ml	0·000 64	1 ml	25 ml	0·008
v)	Linolenic acid and its esters, linseed oil, etc, and derived fatty acids	2 ml then 1 ml(A)	25 ml(A) 25 ml	0·000 64	2 ml then 1 ml(A)	25 ml(A)	0·000 64
vi)	Tall oil fatty acids	Dilutions should be prepared as given for linoleic acid and its esters, etc, but a correction should be made, by subtracting from the calculated linoleic acid content half the percentage of resin acids found.					

EXPLANATORY NOTE

This standard was first published in 1971. In the preparation of this revision, considerable assistance has been derived from the recent literature on infant nutrition as well as the Indian National Code for Protection and Promotion of Breast-Feeding, Ministry of Social Welfare, Government of India; Recommended International Standards for Foods for Infants and Children (Codex Alimentraius Commission) and Recommended Dietary Intakes for Indians (ICMR). Physiological significance of different levels/sources of nutrients have been given due consideration in context with the organ/enzymic system especially during the first three months of infancy. This revision includes a number of changes in the requirements, the prominent ones are as follows:

a) The requirement for protein has been lowered in view of recent concepts in nutrition,

b) The requirements for moisture and acid insoluble ash have been modified,

c) The bacteriological specifications have been made more stringent, and

d) Use of several nutritionally important vitamins and minerals has been allowed as optional ingredients.

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS : 2-1960 'Rules for rounding off numerical values (*revised*)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

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